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*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/149923> since 2018-01-21T11:04:35Z

*Published version:*

DOI:10.1016/j.vetimm.2014.02.008

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# UNIVERSITÀ DEGLI STUDI DI TORINO

***This is an author version of the contribution published on:***

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MYCOLOGICAL PROGRESS (ISSN:1617-416X), pp. 575- 588. Vol. 12. 10.1007/s11557-012-0865-3

***The definitive version is available at:***

*La versione definitiva è disponibile alla URL:*

*[<http://link.springer.com/article/10.1007%2Fs11557-012-0865-3>]*

# **Strobilomyces echinocephalus sp. nov. (Boletales) from south-western China, and a key to the genus Strobilomyces worldwide**

Matteo Gelardi, Alfredo Vizzini , Enrico Ercole, Samuele Voyron, Gang Wu and Xing-Zhong Liu

## **Abstract**

*Strobilomyces echinocephalus* Gelardi & Vizzini sp. nov. is described as a new species, based on collections from Kunming (Yunnan Province, China). Macro-morphological and micro-morphological features, ecological data and analyses of RPB1 and ITS2 sequences support the establishment of this new taxon. Colour pictures of fresh basidiomata in habitat, scanning electron microscope (SEM) micro-photographs of the spores, and a line-drawing displaying the most relevant anatomical characters are provided in this paper. *Strobilomyces zangii* Gelardi nom. nov. is proposed to replace the name *Heimiella nigricans* M. Zang. A dichotomous key to the *Strobilomyces* species at global level is also provided at the end of this work.

## **Introduction**

Towards the end of 2011, the first author had the opportunity to undertake a mycological expedition to China, with the main aim of collecting and studying East Asian boletoid mushrooms. During field sampling around Kunming, Yunnan Province, various basidiomata of a remarkable species of *Strobilomyces* Berk. were collected in a pure stand of *Castanopsis orthacantha* Franch. Detailed examination of the morphological features revealed that the fungus did not belong to any of the known species occurring in China, nor to those as yet reported from Japan and south-eastern Asia. We failed to identify our collection, despite a thorough search through the available literature. None of the taxa of *Strobilomyces* matched the peculiar characteristics of this Chinese species.

The molecular phylogenetic trees inferred from the partial sequences of the largest subunit of RNA polymerase II (RPB1) and from the ITS2 regions of the rDNA support and confirm the independent status of this species, as well as its differentiation from other related taxa. Herewith, *Strobilomyces echinocephalus* is proposed as a new species, and a detailed description is provided, along with a comparison with the closest taxa. In addition, based on morphological features of the type specimen, *Strobilomyces zangii* Gelardi nom. nov. is proposed to replace the name *Heimiella nigricans* M. Zang, and a dichotomous key to the *Strobilomyces* species worldwide is also provided.

## **Materials and methods**

### **Morphology**

Macroscopic description, macro-chemical reactions, habitat notations and associated plant communities were based upon detailed field notes of fresh basidiomata, colours being described in general terms only. Micro-morphologic features were observed on dried

material; sections of this dried material were revived in water, 5 % potassium hydroxide (KOH), or in ammoniacal Congo red. The observation of the structure and measurement of the anatomical features (excluding spores) were performed by mounting the preparation in ammoniacal Congo red; the colour and the amount of pigmentation was described after examination in water and 5 % KOH mounts. Measurements were made at 1000× magnification with a calibrated ocular micrometer. Spore dimensions were taken from the hymenophore of mature specimens and given as (minimum) average ± standard deviation (maximum), Q = average quotient (length/width ratio) ± standard deviation, while average spore volume was approximately estimated as a rotation ellipsoid ( $V = 4/3 * (\text{length}/2) * ((\text{width}/2) * \text{width}) * \pi/2 \pm \text{standard deviation}$ ). The notation [88,3,3] indicates that measurements were made on 88 spores in three samples from three collections. Amyloid reaction was also tested by staining the spores in Melzer's reagent. Anatomical features were observed on glass slides with a Nikon Eclipse E200 optical light microscope, and all line-drawings of microstructures were made from rehydrated material. Specimens of the type collection examined in this study were deposited in TO and KUN-HKAS. Herbarium acronyms follow Thiers (2011). Author citations follow the Index Fungorum—Authors of Fungal Names (<http://www.indexfungorum.org/authorsoffungalnames.htm>).

## Scanning electron microscope photographs

The hymenophoral material of *Strobilomyces echinocephalus* for scanning electron microscope (SEM) analysis was taken from the dried KUN-HKAS 74586 (Topotype collection). It was attached to stubs using double-side adhesive tape and then coated with gold using an ion-coater. After gold sputtering, the material was examined and photographed under a Hitachi S4800. To check spores ornaments of *S. echinocephalus*, its holistic, dorsal & ventral views were scanned at the 2000× and 10000× magnification mode, separately.

## DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was extracted from 1 mg of herbarium specimen (TO MG001, Holotype), by using the DNeasy Plant Mini Kit (Qiagen, Milan Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990; Gardes and Bruns 1993). The RPB1 region was amplified by nested PCR. For the first round of PCR, RPB1A-st1 forward and RPB1C-st1 reverse primers were used (Sato et al. 2011). The first PCR was done under the following conditions: 95 °C for 5 min, followed by 40 cycles of 30 s at 95 °C, 30 s at 53 °C and 60 s at 72 °C, followed by a final extension of 7 min at 72 °C. For the second round of PCR, two different amplification reactions were set up, one with RPB1A-st2 forward primer and RPB1C-st4 reverse primer, and a second one with RPB1A-st4 forward primer and RPB1C-st2 reverse primer (Sato and Murakami 2008). The PCR reactions were done under the following conditions: 95 °C for 5 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 60 °C and 72 °C for 45 s followed by a final extension of 7 min at 72 °C. Amplification reactions were performed in a T3000 Thermocycler (Biometra, Goettingen, Germany). The PCR products were purified with the QIAquick PCR Purification Kit (Qiagen, Milan, Italy) following the manufacturer's instructions, and sequenced by MACROGEN Inc. (Seoul, Republic of Korea). The sequences were assembled and edited with Geneious v5.3 software suite (Drummond et al. 2010). The sequences were submitted to GenBank and their accession numbers are reported in Figs. 1 and 2.

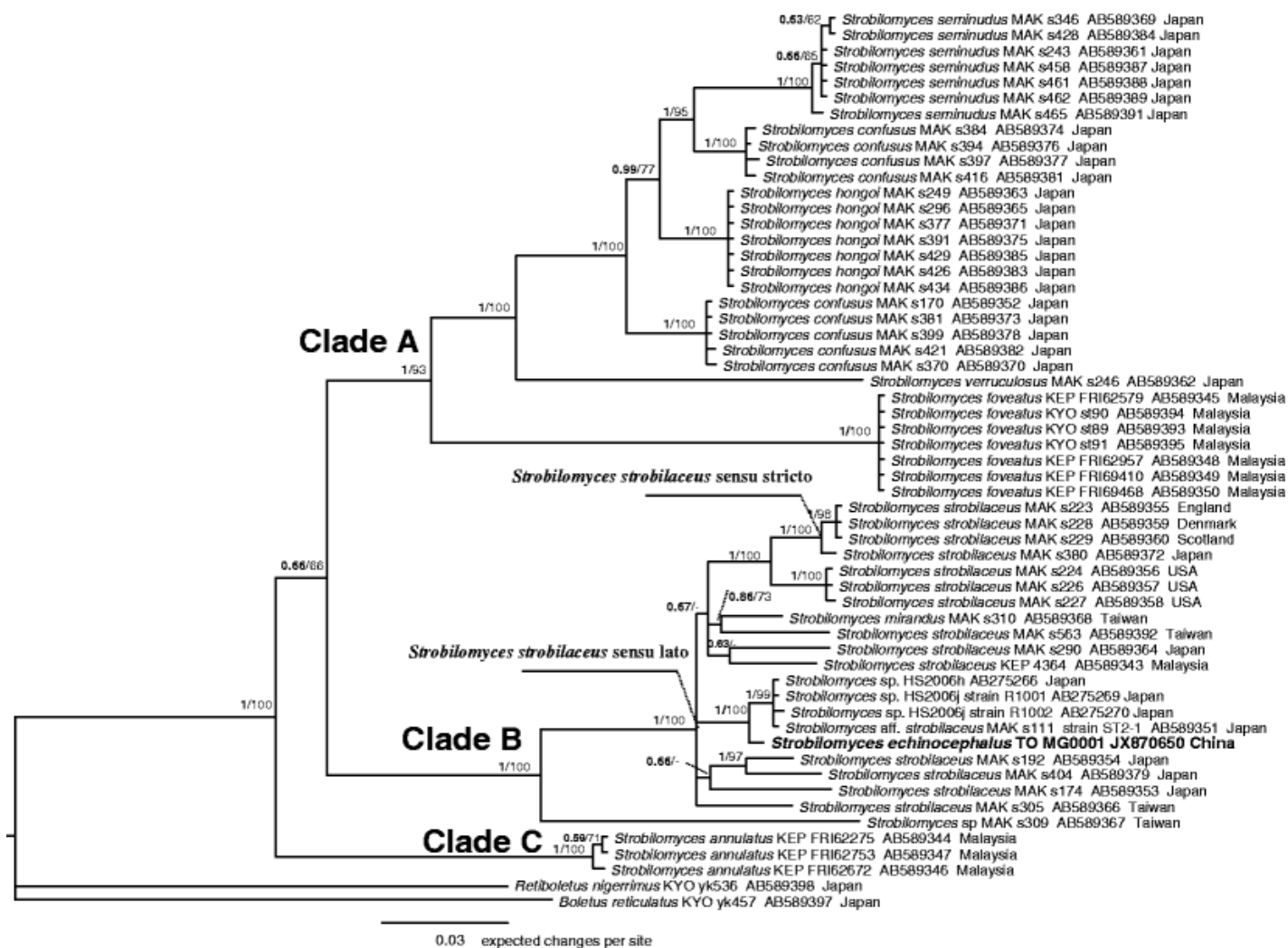


Fig. 1  
Bayesian phylogram obtained from the general RPB1 sequence alignment. *Retiboletus nigerrimus* and *Boletus reticulatus* were used as outgroup taxa. Support values in either the Bayesian (Posterior Probabilities values [BPP]) or maximum likelihood (ML Bootstrap percentage [MLB]) analyses are indicated. Only BPP values over 0.50 and MLB values over 50 % are given above clade branches

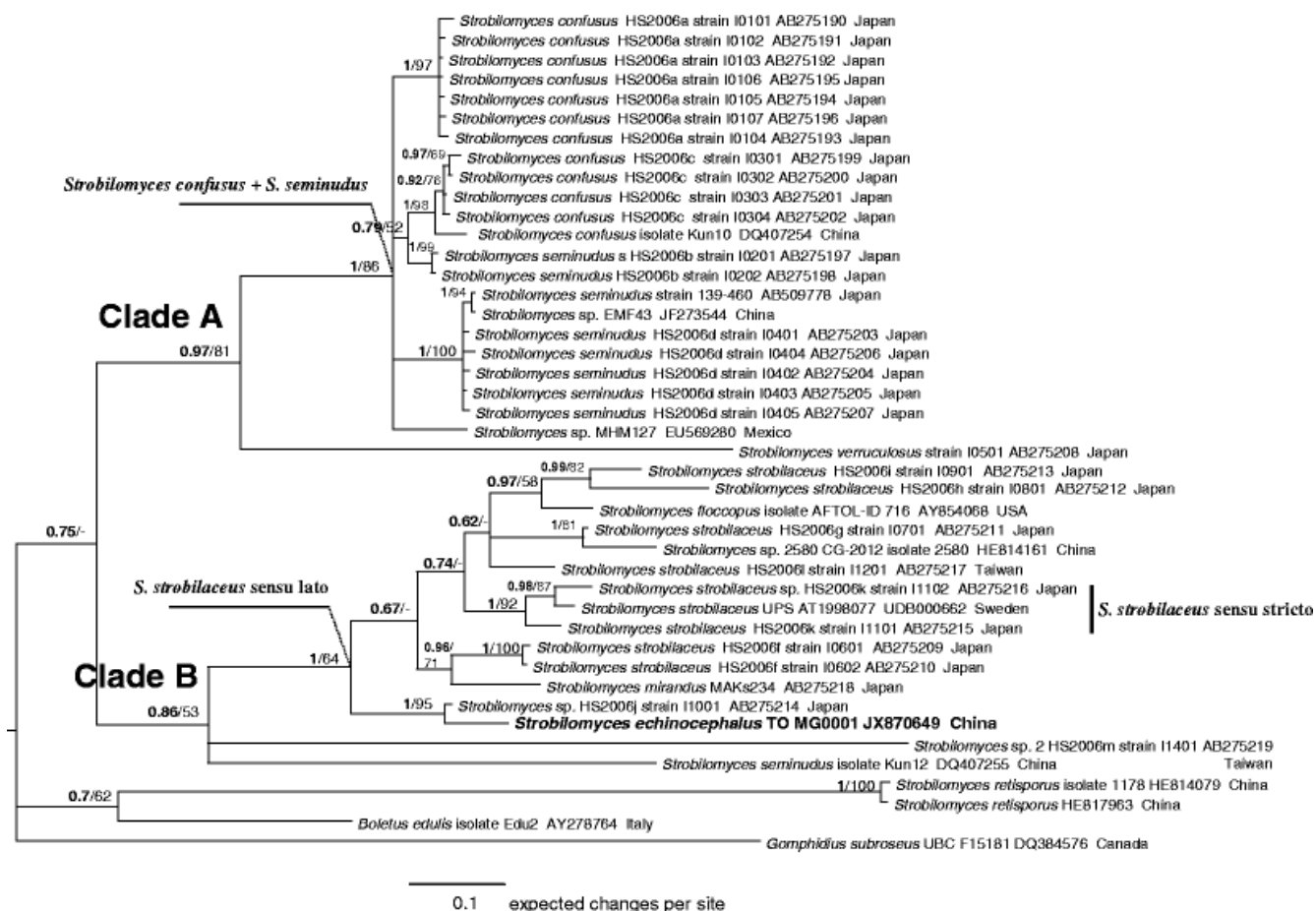


Fig. 2

Bayesian phylogram obtained from the general ITS2 sequence alignment. *Boletus edulis* and *Gomphidius subroseus* were used as outgroup taxa. Support values in either the Bayesian (Posterior Probabilities values [BPP]) or maximum likelihood (ML Bootstrap percentage [MLB]) analyses are indicated. Only BPP values over 0.50 and MLB values over 50 % are given above clade branches

## Sequence alignment and phylogenetic analysis

The sequences obtained in this study were checked and compared to those available in the GenBank database (<http://www.ncbi.nlm.nih.gov/Genbank/>) using the blastn algorithm. Based on the blastn results, sequences were selected from GenBank and UNITE (<http://unite.ut.ee/>), according to the outcomes of recent phylogenetic studies on *Strobilomyces* (Sato et al. 2007, 2011). Separate analyses of RPB1 and ITS2 sequences, and phylogenetic framework were carried out according to Sato et al. (2007, 2011). Alignments were generated using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The sequence alignments were refined manually with MEGA 5.0 (Tamura et al. 2011).

The best-fit models were estimated by both the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) using jModelTest 0.1.1 (Posada 2008) to provide a substitution model for each single alignment. Molecular phylogenetic analyses were performed using the Bayesian inference (BI) and maximum likelihood (ML) approaches. For all phylogenetic analyses, the RPB1 data sets were divided into three partitions (two coding regions and one non-coding) (Sato et al. 2007). According to Sato et al. (2007, 2011), *Retiboletus nigerrimus* (AB589398)—*Boletus reticulatus* (AB589397) and *Boletus edulis* (AY278764)—*Gomphidius subroseus* (DQ384576) were chosen as outgroup taxa for the RPB1 and ITS2 data set, respectively.

BI using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Four incrementally heated simultaneous MCMC were run over 10,000,000 generations, under model assumption. Trees were sampled every 1000 generations, resulting in an overall sampling of 10,001 trees. The “burn-in” value was evaluated using Tracer 1.5 (Rambaut and Drummond 2007). The first 20 % of trees were discarded as “burn-in”. For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). ML estimation was performed through RAxML v.7.3.2 (Stamatakis 2006) with 1,000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm (for all partitions, respectively) to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the “-f a” option of RAxML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm. BI and ML analyses were run on the CIPRES Science Gateway web server (Miller et al. 2010). Only BPP values over 0.50 and MLB over 50 % are reported in the resulting trees (Figs. 1 and 2).

## Results

### Phylogenetic analysis

The RPB1 data set comprised 57 taxa and 1,038 characters, and contains 403 variable sites. The evolutionary model used for the coding regions were SYM + G, while GTR + G for the non-coding region. The ITS2 data set comprised 43 taxa and 590 characters, and contains 386 variable sites.. The evolutionary model used for this region was GTR + G.

BI and ML trees were congruent and thus, only Bayesian trees with both BPP and MLB values are shown in Fig. 1 and Fig. 2. The phylogenetic species recognized by Sato et al. (2007, 2011) are also recovered in our sequence analyses (Figs. 1 and 2). Three major clades were distinguished within *Strobilomyces* according to the RPB1 analysis (Fig. 1): clade A (BPP =1, MLB = 93 %), clade B (BPP =1, MLB = 100 %), and clade C (BPP =1, MLB = 100 %). Clades A and B are also recovered by ITS2 analysis (Fig. 2).

*Strobilomyces echinocephalus* falls, in both the RPB1 and IT2 analysis, into the clade B where it is sister (BPP =1, MLB = 100 % in the RPB1 analysis; BPP =1, MLB = 95 % in the ITS2 analysis) to collections of an undescribed *Strobilomyces* species from Japan (named by Sato et al. 2007, 2011 as *Strobilomyces* sp. or *Strobilomyces* aff. *strobilaceus*).

The two ITS sequences of *Strobilomyces retisporus* (Pat. & C.F. Baker) X.L. Mao retrieved from GenBank (HE814079 and HE817963) fall outside *Strobilomyces*. *Boletus retisporus* Pat. & C.F. Baker is, according to Horak (2004), the type of *Heimioporus* E. Horak, a genus comprising species with a *Xerocomus*-like habit and olive-brown spores, which are usually alveolate-reticulate to reticulate or with pit-like perforations.

### Taxonomy

*Strobilomyces echinocephalus* Gelardi & Vizzini sp. nov. (Figs. 3 and 4)  
Mycobank MB 801553

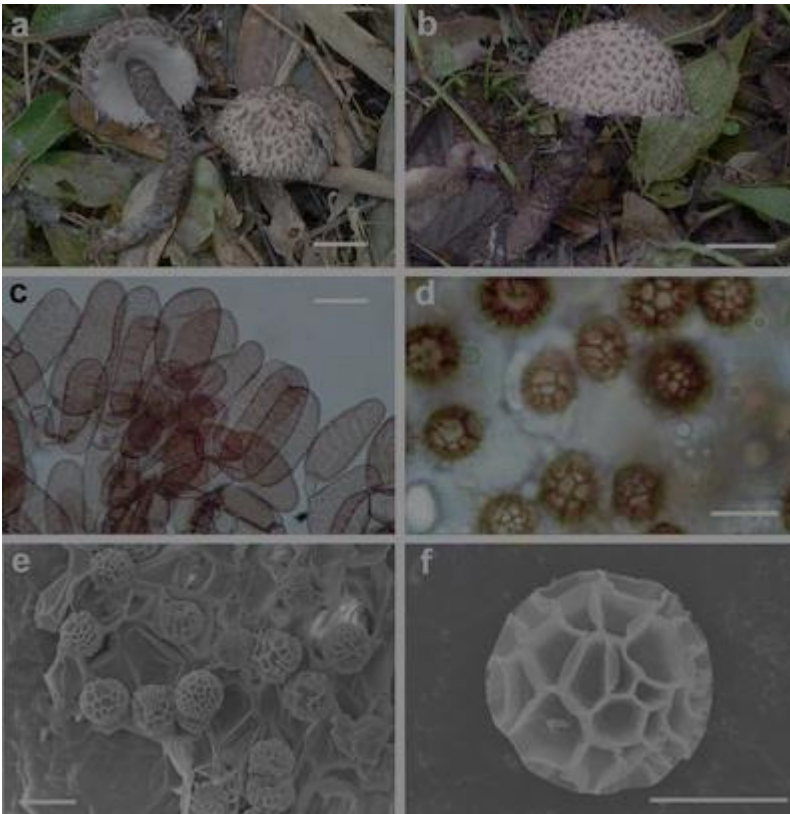


Fig. 3  
*Strobilomyces echinocephalus*. a Basidiomata (TO MG001, holotype). b Basidioma (KUN-HKAS 74586, topotype). c Elements of the stipe-covering flocci in water (TO MG001, holotype). d Spores in 5 % KOH (TO MG001, holotype). e, f Spores under SEM (KUN-HKAS 74586, topotype). Scale bars a, b = 2 cm; c = 20  $\mu$ m; d, e = 10  $\mu$ m; f = 5  $\mu$ m. Photos a-c-d by M. Gelardi, b-e-f by G. Wu

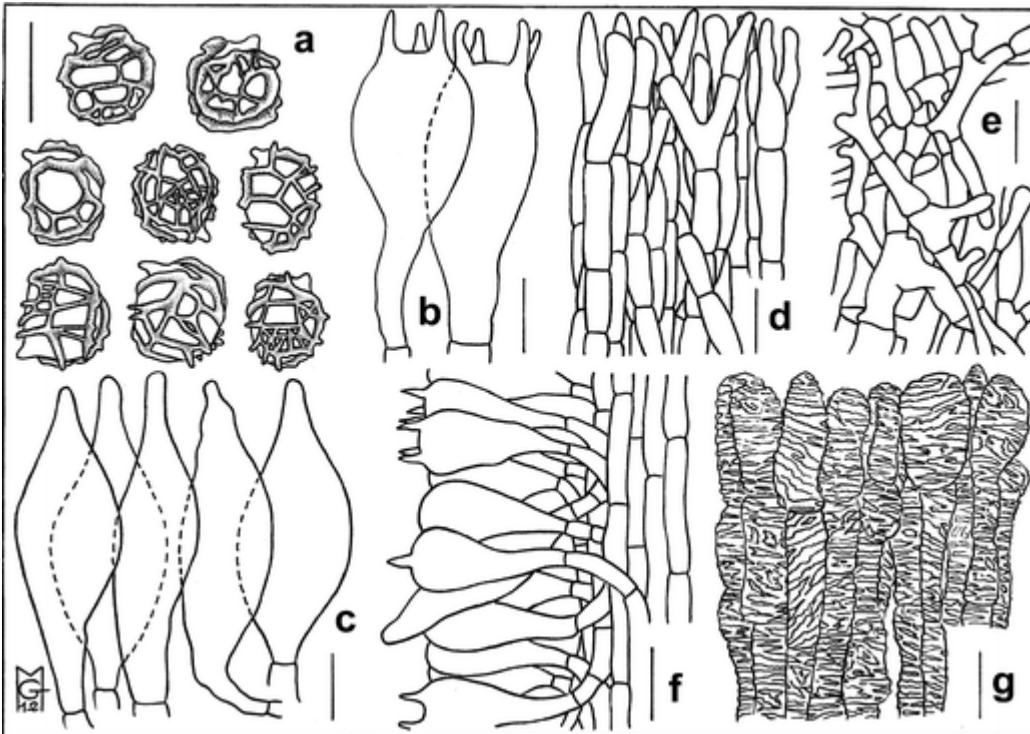


Fig. 4  
*Strobilomyces echinocephalus*. Microscopic features (from TO MG001, holotype). a Spores. b Basidia. c Pleurocystidia and cheilocystidia. d Pileipellis. e Marginal veil. f Stipitipellis. g Stipe-covering flocci. Scale bars a-c = 10  $\mu$ m; d-g = 20  $\mu$ m. Line drawing by M. Gelardi



### *Etymology*

The specific epithet consists of two particles deriving from the Greek adjective *εκεῖνος* “ekinos” (thorny, keen) and the Greek word *κεφάλι* “kefáli” (head), referring to the thorny pileus of the basidiomata.

### *Original diagnosis:*

Basidiomata small to medium-sized and slender; pileus surface ornamented throughout by thin, soft and scattered, blackish-brown scales, at first uplifted and pointed then tending to repent and finally laying upon the whitish ground surface; margin appendiculate with membranaceous, blackish velar remnants. Hymenophore tubular, whitish to grey, bruising rusty-brown then blackish. Stipe longitudinally striped, pale grey, densely covered by cottony to woolly, blackish-brown flocci rising in fluffy tufts; basal mycelium whitish, rhizomorphs blackish. Context whitish, but grayish in the stipe and increasingly darkening downwards, turning unevenly grayish-black straightaway within few minutes from cutting. Spores small-sized, (6.7) 8.0–11.5 (12.8) × (6.1) 6.8–9.9 (11.5)  $\mu\text{m}$  including ornamentation, broadly ellipsoid to subglobose, pale brown in water and KOH, ornamented by a complete and uninterrupted, high reticulation. Clamp connections absent.

Habitat: in subtropical montane environment, among the grass under *Castanopsis orthacantha*.

Holotypus: China, Yunnan Province, Qiongzhu Temple (Kunming), 2150 m.a.s.l., 5 October 2011; legit. M. Gelardi, B. Feng, G. Wu and Y.- J. Hao, collection deposited in TO MG001.

### *Macro-characters*

Ontogenetic development, likely metavelangiocarpic and stipitocarpic according to Reijnders (1948), Singer (1986), Watling (2008), for the genus *Strobilomyces* as a whole. Pileus 4.9–5.2 (5.4) cm. broad, long lasting convex then progressively flattening, regularly to hardly unevenly shaped, moderately fleshy, soft; margin curved downwards, even to faintly waving, exceeding beyond the tubes up to 7 mm, owing to the presence of membranaceous, blackish velar remnants hanging at the pileal edge; surface dry, matt, with a whitish ground colour, entirely beset with thin and sparse, sooty-brown to blackish, erect and acute, pointed scales, up to 5 mm long × 4 mm wide at the base, tending to repent with age or on handling and eventually lying on the surface; ground layer slightly and slowly changing to very pale rose on pressure; subcuticular layer whitish. Tubes somewhat broad, as long as the pileus context thickness or a little longer (up to 0.9 cm high), depressed around the stipe apex and shortly decurrent with a long tooth, whitish at first, then gradually greyish to slate-grey, fading pinkish-brown to rusty brown and finally becoming blackish on cutting. Pores with a concave surface, moderately wide at maturity (up to 1.0 mm in diam.), roundish to slightly angular, simple, concolorous with the tubes and quickly staining pale brown then dark brown to blackish when injured; in the young specimens the hymenophore is hidden by a thin, membranaceous, blackish universal veil soon disrupting in small fragments, which remain appendiculate at pileus margin. Stipe 7.4–10.8 × 0.7–0.9 cm, decidedly longer than the width of the pileus, its length being nearly twice the pileus diameter, slender, central to slightly off-centre, solid, fibrous, dry, straight or curved, equal to faintly attenuated at the apex, roundish at the base, not rooting; pale grey and striate at apex due to the decurrence of the tubes but not reticulate, elsewhere nearly entirely covered by showy, cottony or woolly, sooty-brown to blackish floccules rising in fluffy tufts from the longitudinally striped, underlying grayish-white stipe surface; ring absent; glabrous and fibrillous at the extreme base and covered by an off white basal mycelia tomentum; bruising dingy brown to blackish throughout; rhizomorphs blackish. Context soft in the pileus but decidedly fibrous and firm in the stipe, up to 1.3 cm thick in the center of the pileus, whitish but pale grayish to slate-grey in the stipe and further darkening downwards up to black at the extreme base, turning irregularly grayish then blackish in patches within few minutes after exposure without passing for pinkish or reddish-

rust tones. Smell not very pronounced, faintly fruity. Taste mild. Exsiccate drab brown to fuscous-brown coloured, pileus coarsely tuberculate. Spore print not obtained. Macrochemical reactions: 5 % KOH: reddish-pink on the entire basidiome, both externally and internally.

#### *Micro-characters*

Spores (Figs. 3d-e-f and 4a) (spore body excluding ornamentation) [88,3,3] (6.2)  $8.5 \pm 1.03$  (10.8)  $\times$  (5.6)  $7.1 \pm 0.79$  (9.5)  $\mu\text{m}$ ,  $Q = 1.18 \pm 0.09$ ,  $V = 235 \pm 80.0 \mu\text{m}^3$  in water [including ornamentation: (6.7) 8.0–11.5 (12.8)  $\times$  (6.1) 6.8–9.9 (11.5)  $\mu\text{m}$ ], subglobose to more frequently broadly ellipsoid, rarely globose, both in face and side view, with a pronounced apiculus and with rounded supra-apicular region, moderately thick-walled (0.4–0.8  $\mu\text{m}$ ), having a single, central oil drop at maturity, pale brown in water and 5 % KOH, markedly ornamented by high, dark brownish-black, confluent crests, 0.5–2.0  $\times$  0.3–0.8  $\mu\text{m}$ , merging each other in meshes and forming a well-defined, uninterrupted high reticulate pattern delimiting irregular alveoles; inamyloid in Melzer's reagent. Basidia (Fig. 4b) (30) 33–46 (48)  $\times$  (11) 13–17 (18)  $\times$  3–7  $\mu\text{m}$  ( $n = 17$ ), cylindrical-clavate to truly clavate, moderately thick-walled (0.5–1.0  $\mu\text{m}$ ), frequently 4-spored but also 1-, 2- or 3-spored, showing rather prominent sterigmata, hyaline in water but usually with straw yellow oil guttules and often containing amorphous pale brownish vacuolar sap, without basal clamps; basidioles clavate. Cheilocystidia (Fig. 4c) (36) 40–53 (59)  $\times$  (12) 15–21  $\mu\text{m}$  ( $n = 12$ ), infrequent, straight or sometimes flexuous, ventricose-fusiform to lanceolate, with a relatively short, tapering neck and acute or rounded apex, smooth, moderately thick-walled (0.7–1.0  $\mu\text{m}$ ), hyaline in water and often filled with amorphous pale brownish vacuolar sap. Pleurocystidia (Fig. 4c) (33) 39–61 (69)  $\times$  (11) 12–19  $\mu\text{m}$  ( $n = 11$ ), similar in shape, colour and size to cheilocystidia, rather rare. Hymenophoral trama bilateral-divergent of the "Boletus-type", with distinctly divergent and loosely arranged, gelatinized hyphae (lateral strata hyphae in transversal section not touching and (2) 4–7 (11)  $\mu\text{m}$  distant from each other), hyaline in water and 5 % KOH. Mediostratum 30–50  $\mu\text{m}$  thick, consisting of a tightly adpressed, not gelatinous bundle of hyphae, 4–16  $\mu\text{m}$  broad; in Congo red the mediostratum is darker than the lateral strata. Pileal surface (Fig. 4d) (pileipellis scales) a (physalo) palisadoderm consisting of initially erected and subparallel, short to moderately slender, cylindrical, not or only rarely branched hyphae typically restricted at septa, tending to repent with age and thus turning into a cutis; terminal elements somewhat slender, filamentous, cylindrical but papery towards the tip, with a roundish or more rarely pointed apex, (4) 5–13.5 (14)  $\mu\text{m}$  broad and up to 105  $\mu\text{m}$  long, pale grey in water and 5 % KOH, thick-walled (up to 0.8  $\mu\text{m}$ ); subterminal elements similar in shape, size and colour with terminal ones; terminal and subterminal elements smooth to very vaguely encrusted by a granular, pale grey-brown pigment in water and 5 % KOH. Marginal veil (Fig. 4e) consisting of hyphae similar to those of the pileipellis but on average more slender, filamentous, rather long and sinuous, strongly interwoven and often branched, narrowing at septa, 4–15  $\mu\text{m}$  broad, thick-walled (up to 0.8  $\mu\text{m}$ ), smooth to very vaguely encrusted by a hardly detectable, granular, pale grey-brownish pigment in water and 5 % KOH. Stipital surface (Fig. 4f) (stipitipellis) a texture of slender, parallel to loosely intermingled and longitudinally arranged, adpressed hyphae, (2.5) 3–9 (10)  $\mu\text{m}$  broad, hyaline in water and 5 % KOH; the stipe apex is covered by a well developed caulohymenial layer, with caulobasidioles, scattered caulocystidia and fertile caulobasidia, similar in shape, colour and size with the hymenial ones; lateral stipe stratum under caulohymenium absent. Stipe-covering flocci (Figs. 3c and 4g) consisting of elements very variable in length and shape, cylindrical or ventricose-fusiform to cystidioid, clavate, acorn-shaped, bullet-shaped to subspherical, (5) 7–30 (31)  $\mu\text{m}$  broad, thick-walled (up to 1.2  $\mu\text{m}$ ), pale grey in water and 5 % KOH and strongly encrusted by a "zebra-pattern", pale brownish encrustation. Stipe trama made up by longitudinal and densely arranged, subparallel to moderately interwoven,

filamentous, smooth, inamyloid hyphae, 3–19  $\mu\text{m}$  broad, showing a yellow-brownish content in Melzer's reagent. Rhizomorphs consisting of subparallel to loosely interwoven, sometimes branched, filamentous, smooth-walled hyphae, 3–13  $\mu\text{m}$  broad, pale brownish-grey in water and 5 % KOH. Clamp connections none. Hyphal system monomitic.

#### *Habitat*

Gregarious, in subtropical montane environment on a slight slope facing north, with much vegetation at ground level, including grasses and dry leaves, at the edge of a pathway close to the junction point between a pure stand of *Castanopsis orthacantha* and an unimproved, grass-covered clearing, on acid soil. Other species found in the same habitat: *Agaricus* spp., *Amanita* spp., *Boletus brunneissimus* W.F. Chiu, *Cordyceps militaris* (L.) Link, *Cortinarius* spp., *Fistulina* sp., *Lactarius* spp., *Lepiota* sp., *Mycena* sp., *Russula* spp., *Scleroderma* sp., *Tylopilus virens* (W.F. Chiu) Hongo, *Xerocomus* sp., etc.

#### *Known distribution*

Only from the type locality (Yunnan Province, China).

#### *Examined material*

China, Yunnan Province, Qiongzhu Temple (Kunming), N 25°4.132', E 102°37.379', 2150 m a.s.l., 5 October 2011; one middle-aged and another mature basidiomata fruiting within 1 m of one another, legit. M. Gelardi, B. Feng, G. Wu and Y.- J. Hao (TO MG001, Holotype; KUN-HKAS 75765, Isotype); ibidem, 5 October 2011; one middle-aged basidiome, legit. G. Wu, M. Gelardi, B. Feng and Y.- J. Hao (KUN-HKAS 74586, Topotype).

## Discussion

### *Strobilomyces* and infrageneric clades

As already highlighted by Sato et al. (2011), our analyses also singled out three major clades in *Strobilomyces* (Fig. 1). Clade A (*S. confusus* s.l., *S. hongoi*, *S. seminudus*, *S. verruculosus* and *S. foveatus*) is characterized by spores without a reticulate ornamentation; clade B (*S. strobilaceus* s.l., namely *S. "strobilaceus"* from various locations, *S. echinocephalus* and *S. mirandus*) is distinguished by a clearly reticulate spore ornamentation; and clade C consists only of *S. annulatus*, a Malaysian species circumscribed by an ample, membranaceous ring, and verrucose to subcristate spores (Corner 1972). Spore ornamentation type seems to be a phylogenetically more significant marker than previously expected (Petersen et al. 2011). According to Sato et al. (2007, 2011) and Sato and Murakami (2008, 2009), *S. confusus* and *S. strobilaceus* s.l. (collections from various world locations) comprise several, and probably reproductively isolated, distinct lineages. Our phylogenetic data (Figs. 1 and 2) agree with this conclusion, highlighting the polyphyletic status of these collections when identified solely on morphological basis. Sequences of *Strobilomyces strobilaceus* could be grouped into several lineages (Figs. 1 and 2), probably representing independent species. *Boletus strobilaceus* was originally described by Scopoli (1770) from Hungary. After having selected a lectotype and designated an epitype for this taxon, Petersen et al. (2012) proved the existence of only a single *Strobilomyces* species in Europe, *S. strobilaceus*, on the basis of morphological and ITS data. Therefore, only sequences derived from European collections and those phylogenetically allied with them should be regarded as *Strobilomyces strobilaceus* s.s (Figs. 1 and 2).

## ***Strobilomyces echinocephalus* and allied species**

According to morphological data and phylogenetic analyses of RPB1 and ITS2 sequences, our collection from China should be regarded as an independent species, named as *S. echinocephalus*.

As pointed out previously (see Results), three undetermined collections of the clade B from Japan (HS2006h, HS2006j and MAK s111) are sister to *S. echinocephalus* in the RPB1 analysis (Fig. 1); collection HS2006j is also sister to *S. echinocephalus* in the ITS2 analysis (Fig. 2). Future morphological analyses and molecular work, including additional gene sequences, could provide evidence for considering these collections conspecific to our new species; at the moment data are still insufficient to draw this conclusion.

*Strobilomyces echinocephalus* is clearly characterized and distinguished from the other species of the genus by the following, unique set of macro-morphological, anatomical and edaphic features: (1) small to medium size and slender build; (2) pileus surface whitish and entirely ornamented by thin and scattered, rather soft, blackish-brown spiny scales, initially uplifted and pointed then laying upon the surface with age or on handling; (3) hymenophore (both tubes and pores) bruising rusty-brown then fading to blackish on exposure or when injured; (4) stipe longitudinally striped and almost completely covered by cottony to woolly, blackish-brown flocci gathering in fluffy tufts; (5) context turning grayish-black erratically within few minutes after cutting, but without passing through reddish or rusty hues; (6) basal mycelium whitish; (7) rhizomorphs blackish; (8) spores relatively small, broadly ellipsoid to subglobose, ornamented by a prominent, complete and uninterrupted reticulum; and (9) growing in putative ectomycorrhizal association with *Castanopsis orthacantha* Mast.

When the first author came across that species, he supposed it to be the common *S. confusus* Singer, a species which is widely distributed, not only in North and Central America (Singer 1945, 1970; Smith and Thiers 1971; Villarreal and Perez-Moreno 1989; Both 1993; Bessette et al. 2000; Ortiz-Santana et al. 2007), but also across the Far East (Ying and Ma 1985; Zang 1985, 1997; Imazeki and Hongo 1989; Chang and Mao 1995; Li and Song 2000; Chantorn et al. 2007; Sato et al. 2007; Seehanan and Petcharat 2008; Mao 2009). *S. confusus* has recently indicated by molecular analysis, along with the closely related *S. seminudus* Hongo, as a collective polyphyletic species (Sato et al. 2007, 2011; Sato and Murakami 2008; and our data); however, some macro-morphological discrepancies and further observation under the microscope definitely confirmed it was somewhat different. In fact, the collections showed broadly ellipsoid to subglobose spores with a complete, uninterrupted reticulation, which is completely different from the verrucose to subcristate ornamentation of *S. confusus* s.l. spores. In addition, the larger pileus, the context quickly turning reddish-orange and eventually fading to black on exposure, the presence of an annulus in the upper part of the stipe and the apex slightly reticulate further distinguish this species from *S. echinocephalus* (Singer 1945; Snell and Dick 1970; Smith and Thiers 1971; Weber and Smith 1985; Phillips 1991; Both 1993; Bessette et al. 2000; Kuo 2005; Chantorn et al. 2007; Ortiz-Santana et al. 2007; Seehanan and Petcharat 2008).

From the morphological viewpoint, the most closely related species is undoubtedly the Chinese taxon *S. atosquamosus* J.Z. Ying & H.A. Wen, also recorded in Yunnan Province. However, this species, although outwardly similar, differs from *S. echinocephalus* by the smaller dimension and stocky build, the more crowded and smaller scales on the pileus, the nearly smooth stipe, and anatomically by the decidedly smaller cheilocystidia (Wen and Ying 2001). Even though no information is given concerning the habitat, the many morphological differences suggest they are two different taxa, the non-reddening context and the spore dimension and ornamentation being the only major characters in common.

Amongst the other allied Chinese taxa with spores having a complete reticulum, *S. parvirimosus* J.Z. Ying primarily differs on the basis of the areolate pileus with large and flat patches, but also by the slightly smaller spores and the shorter basidia (Ying 1986), whereas *S. subnigricans* J.Z. Ying and *S. glabellus* J.Z. Ying are easily separated by the blackish to black and glabrous pileus, the almost glabrous stipe and the smaller cheilocystidia (Ying and Ma 1985; Ying 1986). *S. subnigricans* also differs by the more slender stipe, the considerably larger spores and the growth under *Abies* sp. and bamboo plants (Ying 1986).

The dark vinaceous to fuliginous tint of pileus and stipe, the presence of a short reticulum with elongate meshes at the stipe apex, the velar remnants detectable on the upper part of the stipe and the context quickly and evenly turning dull red then darkening on cutting, readily distinguish the Malaysian *S. mollis* Corner from *S. echinocephalus*; conversely, the microscopic features of the two taxa are quite similar (Corner 1972; Horak 2011).

The uncommon, although widespread, single European entity *S. strobilaceus* (Scop.) Berk. is easily discernible from *S. echinocephalus*, solely relying on macromorphological features, as the former is well characterized by the definitely larger dimension, the pileus covered by large and polygonal, imbricate, ascending persistent blackish scales on a nearly concolorous to slightly paler ground surface, the strongly reddening then slowly blackening context on cutting, the less pronounced woolly floccules on the stipe surface, and the presence of a peronate ring and the absence of the whitish basal tomentum; in addition, it shows a somewhat larger spores and cystidia, longer basidia and narrower pileipellis terminal cells (Singer 1967; Alessio 1985; Breitenbach and Kränzlin 1991; Engel et al. 1996; Lannoy and Estadès 2001; Muñoz 2005; Watling and Hills 2005; Knudsen and Taylor 2008; Šutara et al. 2009; Petersen et al. 2012).

### ***Strobilomyces ecology and distribution***

According to Kirk et al. (2008) *Strobilomyces* encompasses some 20 known species worldwide, although the present account, as well as other recent studies (Watling and Li 1999; Sato et al. 2007, 2011; Sato and Murakami 2009) and ongoing research, indicate this genus to be far larger than originally envisaged (Horak, in litt.; Petersen, in litt.), the real number of entities being likely as many as 40. The whole genus is morphologically well delimited and immediately recognizable in the field, due to the squamulose, areolate to shaggy-squarrose appearance of its species, which are usually grayish-brown to blackish throughout, with reddening and/or blackening of fresh tissues exposed to air, the presence of marginal, appendiculate veil, the fuliginous black to purple-blackish spore print, the striking ornamented (echinulate/cristate to completely reticulate), roughly rounded, blackish-brown spores, the basidiomata hardly decomposing, but tending to mummify in habitat, and the mycorrhizal association mainly with hardwoods of the families Fagaceae, Myrtaceae and Dipterocarpaceae (Berkeley 1851; Singer 1986; Watling and Hills 2005; Zang 1997; Watling 2008; Halling 2011; Petersen et al. 2011) but also with Pinaceae (Sato et al. 2007; Petersen et al. 2012). It is mainly distributed in the tropics, extending into temperate areas of the Northern Hemisphere with few representatives, frequent in south-eastern Asia (Chiu 1948; Corner 1972; Singer 1986; Zang 1997; Li and Song 2000; Watling 2008; Halling 2011; Wu et al. 2011), with southern and south-western China undoubtedly hosting the largest concentration of members. Molecular phylogenetic studies elucidated that *Strobilomyces* is closely related to *Boletus* L., *Porphyrellus* E.-J. Gilbert, *Tylopilus* P. Karst and *Spongiforma* Desjardin, Manf. Binder, Roekring & Flegel in the Boletaceae (Binder and Hibbett 2006; Desjardin et al. 2009, 2011; Halling et al. 2012; Zeng et al. 2012).

The extralimital *Afroboletus* Pegler & T.W.K. Young, typified by *A. pterosporus* (Singer) Pegler & T.W.K. Young, seems to be mostly restricted to (sub)tropical and equatorial Africa (Pegler

and Young 1981; Heinemann and Rammeloo 1995), even though it has also been reported from northern South America (Singer 1986) and Malaysia (Halling et al. 2007). Geographical distribution apart, this genus, encompassing the species formerly placed by Singer (1945) in sect. *Pterospori* of *Strobilomyces*, differs from this latter by having broadly ellipsoid and longitudinal ribbed spores (Pegler and Young 1981; Ying and Ma 1985; Heinemann and Rammeloo 1995; Watling 2008; Halling 2011), being practically identical to *Strobilomyces* in every other aspect (Singer 1986). Since *Afroboletus* species have never been included in a phylogenetic analysis to date (Binder and Hibbett 2006), until its definitive taxonomic status is clarified, we prefer to retain it as a separate genus and not to treat it in the key proposed in this study; according to our preliminary analyses, two ITS sequences of *Afroboletus luteolus* (Heinem.) Pegler & T.W.K. Young recently deposited in GenBank by Bidartondo and Doring (acc. no. GQ981489, GQ981490) fall outside of the genus *Strobilomyces* (data not shown). Taking into account that a comprehensive molecular approach to *Strobilomyces* worldwide has not yet been attempted, the following dichotomous key is based on both morphological (see also Horak 1980) and molecular data when available. Species recorded by Watling and Gregory (1986) and successfully quoted by Watling and Li (1999) from Australia but not formally described are also integrated for completeness. Spores dimensions are given including ornamentation.

### Key to the genus *Strobilomyces* worldwide

1a:

Spores echinulate or verrucose to (sub)cristate or with irregular and simple warts sometimes forming an incomplete reticulum...2

1b:

Spores with a well defined, complete and uninterrupted reticulum...22

2a:

Spores truly echinulate with crowded, slender conical spines not forming crests, context and hymenophore staining reddish-orange or reddish-pink then black on exposure...3

2b:

Spores verrucose to (sub)cristate sometimes showing an incomplete reticulum, context and hymenophore turning either reddish-orange or reddish-pink then blackish or directly grayish/blackish or unchangeable at all...6

3a:

Pileus ornamented by dark brown to blackish flat patchy scales, 3–7 cm broad, whitish in the cracks, tubes sinuate to subdecurrent, stipe reticulate in the upper third, floccose elsewhere, spores  $8\text{--}10 \times 6\text{--}8 \mu\text{m}$ , occurring in Malaysia but likely widespread throughout south-eastern Asia ...*S. velutipes* Cooke & Massee ss. Corner 1972

3b:

Pileus covered by erect, echinate or conical warts...4

4a:

Stipe without annulus, pileus 7–10 cm broad, covered by dark brown to black, erect echinate or conical warts,  $1.5\text{--}3.0 \times 1.5\text{--}2.5 \text{ mm}$  on a paler ground colour, tubes sinuate, stipe strongly

reticulate in the upper part, verruculose to squamulose downwards, spores  $9-10.5 \times 8-9 \mu\text{m}$ , reported from Malaysia...*S. foveatus* Corner

4b:

Stipe showing an annulus...5

5a:

Pileus 7 cm broad, covered by dark brown erect and pointed warts on a yellow-ochraceous ground colour, stipe reticulate at the apex but squamulose below the ring, spores  $9.5-13 (14.7) \times 6.3-8.3 \mu\text{m}$ , occurring in central Africa...*S. echinatus* Beeli (? = *S. gilbertianus* Heinem. & Rammeloo)

5b:

As above, but with strongly decurrent tubes..."*S. gilbertianus* var. *decurrens*" Heinem. & Rammeloo

6a:

Pileus scurfy, velvety or very faintly verruculose with warts smaller than 1 mm, context and hymenophore staining reddish then black on exposure...7

6b:

Pileus ornamented by either areolate flat patchy scales or erect and spiny scales or large, obtuse-imbricate ascending scales...9

7a:

Pileus 3–12 cm broad, minutely verruculose with warts  $0.3-0.6 \times 0.25-1 \text{ mm}$ , evenly blackish, hymenophore subdecurrent, reticulate at the apex, covered by adpressed tomentose scales elsewhere and showing a cottony annular zone, spores  $9.1-11 \times 9.0-10.9 \mu\text{m}$ , incompletely reticulate, reported from Japan...*S. verruculosus* Hirot. Sato

7b:

Pileus evenly scurfy-roughened or velvety...8

8a:

Pileus scurfy-roughened and blackish, spores  $7-8.5 (9) \times (5) 5.5-6 (7) \mu\text{m}$ , cristate to incompletely reticulate, reported from Australia...*Strobilomyces* sp. 1 (Watling and Li 1999)

9a:

Pileus ornamented by areolate flat patchy scales...10

9b:

Pileus ornamented by erect, conical spiny scales or by obtuse-imbricate ascending scales, hymenophore and context staining reddish then black on exposure...18

10a:

Pileus and stipe dark purple-brown then blackish, pileus 6.5 cm broad, ornamented by hexagonal rough scales at centre, floccose-shaggy towards the margin, tubes adnate, stipe flocculose, hymenophore and context discolouration unknown, spores  $12.5-15 \mu\text{m}$ , subcristate to incompletely reticulate, occurring in southern and south-eastern Asia...*S. nigricans* Berk. (= *S. phaeus* Pat. nom. inval., fide Horak 1980)

10b:

Basidiomata with a different set of characters...11

11a:

Stipe with an annulus or a well defined annular zone or at least with some velar remnants towards the apex, hymenophore and context staining reddish then black on exposure...12

11b:

Stipe without annulus...14

12a:

Stipe completely smooth although showing remnants of the partial veil at apex, pileus 2.8-7.0 cm broad, covered by subtomentose-rimose flattened, blackish areolate patches, 0.3-1.2 cm wide, tubes sinuate, spores  $9.0-11.2 \times 7.6-9.4 \mu\text{m}$ , subcristate, reported from south-western China...*S. areolatus* H.A. Wen & J.Z. Ying

12b:

Stipe ornamented by adpressed tomentose scales or distinctly flocculose...13

13a:

Pileus 3-10 cm broad, glabrous to slightly ornamented by grayish to blackish flat patchy scales 2-10 mm wide on a whitish ground colour, tubes subdecurrent, stipe with a distinct thickened annular zone at apex, reticulate at the extreme apex and covered by adpressed tomentose scales elsewhere, spores  $7-10 \times 6-9 \mu\text{m}$ , subcristate, reported from Japan and China...*S. seminudus* Hongo

13b:

Pileus ornamented by flattened, blackish scales, stipe floccose, spores  $9-11 \times 7-9 \mu\text{m}$ , verrucose to subcristate, reported from Mexico...*S. strobilaceus* var. *mexicanus* R. Heim & Perr. Bertr. nom. inval. (Heim and Perreau-Bertrand 1964)

14a:

Stipe reticulate at least at apex...15

14b:

Stipe not reticulate...16

15a:

Pileus 3-10 cm broad, ornamented by grayish to blackish flat patchy scales 2-8 mm wide, whitish in the cracks, tubes subdecurrent, stipe coarsely reticulate in the upper part, verruculose to squamulose downwards, hymenophore and context staining reddish then black on exposure, spores  $8-10 \times 8.5-9.5 \mu\text{m}$ , subcristate, reported from Japan...*S. hongoi* Hirot. Sato

15b:

Pileus 4.4-8.2 cm broad, covered by subtomentose-rimose flattened, brownish-black areolate patches, tubes sinuate, stipe smooth, reticulate at apex but pruinose elsewhere, hymenophore and context discoloration unknown, spores  $8.1-11.1 \times 7.2-10.8 \mu\text{m}$ , verrucose to subcristate, reported from eastern and south-western China...*S. subnudus* J.Z. Ying



16a:

Hymenophore and context reddening then blackening on exposure, pileus 4.5–5.3 cm broad, ornamented by blackish flattened scales, whitish in the cracks, stipe smooth and minutely fibrillose with basal lilaceous tomentum, spores  $10\text{--}11 \times 8.3\text{--}10 \mu\text{m}$ , verrucose to subcristate, reported from Australia...*Strobilomyces* sp. 3 (Watling and Li 1999)

16b:

Context not reddening on exposure...17

17a:

Pileus 10–15 cm broad, deeply areolate, with blackish flattened patchy scales, tubes sinuate, stipe smooth and longitudinally striate, context not reddening on exposure and possibly unchangeable at all, spores  $8.5\text{--}10 \times 8.5\text{--}9.5 \mu\text{m}$ , verrucose to incompletely reticulate, occurring in south-western China...*S. giganteus* M. Zang

17b:

Pileus 5.5–6 cm broad, chestnut brown and velvety rimose-areolate, pale brown in the cracks, tubes sinuate, stipe smooth, entirely pruinose, context staining blackish straightaway on exposure, spores  $10.1\text{--}12.6 \times 8.6\text{--}10.8 \mu\text{m}$ , verrucose to subcristate, reported from south-western China...*S. velutinus* J.Z. Ying

18a:

Pileus (2.5) 5–7.5 cm broad, ornamented by large, irregular, grey to dark grey obtuse-imbricate ascending scales, tubes sinuate, stipe tomentose and striate at apex, spores (7.5)  $8\text{--}11 \times 5.5\text{--}7.5 \mu\text{m}$ , verrucose to subcristate, reported from Australia...*S. velutipes* Cooke & Masee ss. Watling and Li (1999) (? = *S. indica* Lloyd)

18b:

Pileus ornamented by erect, conical spiny scales...19

19a:

Stipe with annulus...20

19b:

Stipe without annulus...21

20a:

Pileus large, 10–13 cm broad, covered by dark vinaceous-brown, erect, soft and deterrent conical warts,  $2\text{--}4 \times 2\text{--}3 \text{ mm}$ , tubes adnate, stipe shallowly reticulate and densely floccose with an ample, membranaceous ring, spores  $11.5\text{--}13 \text{ (15)} \times 9.5\text{--}11 \text{ (12)} \mu\text{m}$ , verrucose to subcristate, reported from Malaysia...*S. annulatus* Corner

20b:

Pileus 3–12 cm broad, covered by large, blackish-gray, erect conical warts on a whitish to gray ground colour, tubes sinuate, stipe floccose and showing a cottony annulus, slightly reticulate at apex, spores (8.8)  $10.5\text{--}12.5 \times (8.2) \text{ } 9.5\text{--}10.5 \text{ (11.2)} \mu\text{m}$ , verrucose to subcristate, distribution disjunct, occurring in North/Central America and in eastern and south-eastern Asia...*S. confusus* s.l. Singer

21a:

Pileus 4–8 (20) cm broad, covered by densely crowded blackish pyramidal soft warts 2–8 mm wide on a dirty brown ground colour, tubes sinuate, stipe nearly smooth to subsquamulose, spores (9.0) 9.5–13 (14.4)  $\times$  7–10 (11.5)  $\mu\text{m}$ , verrucose to subcristate, occurring in southern and south-eastern Asia, as well as in Indonesia...*S. polypyramis* Hook.

21b:

Pileus up to 9 cm broad, covered by large, erect, blackish-lilac scales tending to recline towards the margin, ground colour whitish, stipe white, woolly at apex, fibrillose-floccose downwards, hymenophore and context discoloration not known with certainty but possibly reddening then blackening, spores broadly ellipsoid, 8.5–10 (11)  $\times$  5.5–7.5  $\mu\text{m}$ , verrucose to cristate, reported from Australia...*Strobilomyces* sp. 2 (Watling and Li 1999)

22a:

Basidiomata golden tawny or golden yellow...23

22b:

Basidiomata differently coloured...24

23a:

Pileus and stipe golden tawny, golden yellow to golden orange, becoming brown to blackish when over mature, velar remnants on pileus margin yellow to dull yellow, pileus (2.5) 3–7 cm broad, ornamented in the discal zone by erect, conical, pointed concolorous warts with fuscous tips, 3  $\times$  3.5 mm, becoming flattened towards the margin, tubes adnexed to subdecurrent, stipe shallowly reticulate in the upper part and showing a woolly feeble annulus, faintly flocculose-squamulose below, hymenophore and context staining reddish then black on exposure, spores (7.5) 8.5–9.5 (10)  $\times$  (7) 7.5–8.5 (9)  $\mu\text{m}$ , occurring across eastern and south-eastern Asia...*S. mirandus* Corner (see also Ge and Yang 2005 and Sato et al. 2005)

23b:

As above, but with smaller pileus (1–4.2 cm broad) and spores (6.5–8.0  $\times$  6.3–7.2  $\mu\text{m}$ ), reported from eastern China (in accordance with Ge and Yang 2005, this species might be conspecific with *S. mirandus*)...*S. sanmingensis* N.L. Huang (Huang 2002)

24a:

Basidiomata at first pinkish to greyish-pink or pinkish-tan, becoming dark brown with age, pileus 3–12 cm broad, ornamented by flattened to faintly pointed scales, tubes slightly decurrent, stipe reticulate above the cottony annulus, floccose elsewhere, hymenophore and context staining reddish then black on exposure, spores 9–12  $\times$  7–9  $\mu\text{m}$ , occurring in North America...*S. dryophilus* Cibula & N.S. Weber nom. inval. (Weber and Smith 1985)

24b:

Basidiomata grayish-brown to blackish throughout...25

25a:

Pileus 5–6 cm broad, ornamented by small, partially overlapping blackish-purple scales, tubes sinuate, stipe smooth, longitudinally striate and without annulus, context not reddening on exposure and possibly unchangeable at all, spores (12) 12.5–15  $\times$  11–13 (13.5)  $\mu\text{m}$ , hyphae showing numerous clamp connections at septa, basidiomata occurring at very high elevation

(around 4000 m a.s.l.), sometimes growing on decaying wood and apparently restricted to conifer woodlands in eastern Himalayas (China)...*S. alpinus* M. Zang, Y. Xuan & K.K. Cheng

25b:

Basidiomata with a different set of characters...26

26a:

Pileus glabrous, without any kind of ornamentation...27

26b:

Pileus ornamented by either areolate flat patchy scales or erect and spiny scales or large and polygonal imbricate, ascending scales...28

27a:

Pileus 9.5 cm broad, wrinkled, blackish, tubes adnate-subdecurrent, stipe reticulate above the remnants of the floccose annulus, smooth below, discoloration of hymenophore and context unknown, spores  $8.8\text{--}10.4 \times 6.6\text{--}8.1\text{ }\mu\text{m}$ , occurring in south-western China....*S. glabellus* J.Z. Ying

27b:

Pileus 6 cm broad, smooth, blackish, tubes adnate, stipe entirely glabrous, without annulus, discoloration of hymenophore and context unknown, spores  $12.6\text{--}15.9 \times 10.8\text{--}12.6\text{ }\mu\text{m}$ , occurring in central China...*S. subnigricans* J.Z. Ying

28a:

Pileus distinctly areolate or with flat patchy scales...29

28b:

Pileus ornamented by erect and spiny scales or large and polygonal imbricate ascending scales...35

29a:

Spores  $13\text{--}15 \times 10\text{--}13.5\text{ }\mu\text{m}$ , pileus 4–5 cm broad, ornamented by flattened, angular, blackish scales on a paler, grayish-brown ground colour, stipe reticulate with elongate meshes, context staining grayish straightaway on exposure, reported from Indonesia and possibly Malaysia and Australia...*S. nigricans* Berk. ss. Boedijn 1960

29b:

Spores decidedly smaller...30

30a:

Tubes distinctly decurrent, pileus up to 10 cm broad, dark rusty brown, glabrous and rimose-areolate, stipe reticulate at the apex, longitudinally streaked and scurfy elsewhere, without annulus, hymenophore and context discoloration unknown, spores  $9\text{--}12\text{ }\mu\text{m}$ , occurring in south-western China...*S. glabriceps* W.F. Chiu

30b:

Tubes sinuate or adnate, spores slightly smaller...31

31a:

Hymenophore greenish when fresh, pileus covered by blackish, flattened velvety scales, stipe smooth but showing an annulus near the apex, context staining grayish straightaway on exposure, spores broadly ellipsoid, (8)  $8.5\text{--}10 \times 6\text{--}7$  (7.5)  $\mu\text{m}$ , reported from Australia...*S. cf. mollis* Corner (see Watling and Gregory 1986; Watling and Li 1999)

32a:

Hymenophore whitish at first then greyish and further darkening, owing to the ripening of the spores...33

33a:

Pileus small, 3–4.5 cm broad, blackish, subtomentose and finely areolate, whitish in the cracks, tubes adnate, stipe with a thickened annular zone at apex, entirely smooth and minutely fibrillose, basal mycelium brownish, context not turning reddish and possibly unchangeable on exposure, spores  $8.5\text{--}10 \times 8\text{--}9$   $\mu\text{m}$ , occurring in south-western China...*S. zangii* Gelardi

33b:

Pileus larger...34

34a:

Pileus 7.7–9.2 cm broad, with large, areolate, flattened blackish patches, 5–18 mm wide, tubes sinuate, stipe reticulate above the floccose annulus, smooth below, hymenophore and context discoloration unknown, spores  $7.4\text{--}8.9 \times 6.7\text{--}8.1$   $\mu\text{m}$ , occurring in southern China...*S. latirimosus* J.Z. Ying

34b:

As above, but pileus with smaller areolate patches, 2–6 mm broad and stipe without annulus, tomentose in the upper half, glabrous elsewhere, hymenophore and context discoloration unknown, spores  $7.9\text{--}9.6 \times 5.8\text{--}7.8$   $\mu\text{m}$ , occurring in south-western China...*S. parvirimosus* J.Z. Ying

35a:

Pileus 6–16 (20) cm broad, covered by large and polygonal, imbricate, ascending persistent blackish scales on a nearly concolorous to slightly paler background surface, tubes sinuate, stipe floccose with a peronate annulus on the upper part, hymenophore and context staining reddish then black on exposure, spores (8.5)  $9.5\text{--}14$  (15)  $\times$  (7.5)  $8.5\text{--}12$  (13)  $\mu\text{m}$ , occurring in Europe and Japan (Sato et al. 2011 and our data), most likely widespread throughout Asia but real distribution still uncertain...*S. strobilaceus* (Scop.) Berk. ss. Auct. Eur. [= *S. floccopus* (Vahl) P. Karst.]

35b:

Pileus ornamented by erect and spiny scales...36

36a:

Context and hymenophore staining reddish then black on exposure, stipe with an annulus or at least with some velar remnants...37

36b:

At least context staining erratically blackish straightaway or possibly unchangeable at all on exposure, stipe without annulus...38

37a:

Pileus 2–7 cm broad, ornamented by dark vinaceous to fuliginous erect, soft conical warts, 5 × 5 mm, tubes sinuate to subdecurrent, stipe reticulate at the apex and showing velar remnants in the upper third, somewhat flocculose-squamulose elsewhere, basal mycelium white, spores 9–10.5 × 7.5–9 µm, reported from Malaysia, but most likely widespread in (sub)tropical Asia...*S. mollis* Corner (? = *S. montosus* Berk.)

37b:

Pileus up to 13.5 cm broad, covered by numerous tough, erect, conical and pointed scales rising from a polygonal base, stipe floccose and with an apical annulus, spores very variable in shape and dimension, reported from Mexico...*S. strobilaceus* var. *zapotecorum* R. Heim & Perr. Bertr. nom. inval. (Heim and Perreau-Bertrand 1964)

38a:

Pileus 4.9–5.2 (5.4) cm broad, ornamented by thin and scattered, blackish-brown erect spiny scales, 5 × 4 mm, standing on a whitish ground colour, tubes sinuate, stipe striped lengthwise and almost completely covered by cottony to woolly, blackish-brown flocci, basal tomentum whitish, hymenophore turning rusty brown then black on bruising, context staining unevenly blackish straightaway after exposure, spores (6.7) 8.0–11.5 (12.8) × (6.1) 6.8–9.9 (11.5) µm, occurring in south-western China and probably in Japan...*S. echinocephalus* Gelardi & Vizzini

38b:

Pileus 5 cm broad, ornamented by blackish small, erect and crowded scales, 2 × 1 mm, tubes sinuate, stipe smooth, very minutely pruinose throughout, context grayish and not reddening on exposure, spores 9.0–11.2 × 8.1–10 µm, occurring in south-western China...*S. atosquamosus* J.Z. Ying & H.A. Wen

## Rejected and doubtful names

According to the type specimens revision carried out by Horak (1980 and in litt.), the following taxa must be either excluded from *Strobilomyces* or putatively synonymized with other species (accepted species that have already formally been transferred to other genera are not listed below):

- *S. coturnix* Bouriquet → *Afroboletus*.
- *S. immutabilis* Bouriquet → *Boletellus*.
- *S. fasciculatus* Cooke → Type immature/sterile, likely a *Boletus*/*Xerocomus* according to the very short protologue.
- *S. indica* Lloyd → No data, but according to Corner (1972), Horak (1980) and Singer (1945), this latter quoted by Watling and Gregory (1986), it might be conspecific with the Australian *S. velutipes*.
- *S. kalimpongensis* Bose → No data, type material not available (*Polyporus* s.l.?).
- *S. montosus* Berk. → Most likely conspecific with the Malaysian *S. mollis*, from which it differs on account of the slightly larger spores, 9–13 µm (Horak 1980), certainly not conspecific with *S. polypyramis* as previously suggested by Boedijn (1951). However, since the taxonomic circumscription of this taxon remains unclear, we prefer to maintain the name *S. mollis* at the expense of *S. montosus*, even though this latter would be chronologically foregoing.

- *S. pauper* Singer → *Afroboletus*, see also Singer (1986).

## Proposal of a new name for *Heimiella nigricans* Zang

Following Sato et al. (2011), there should be one more *Strobilomyces* species initially described by the late Professor Mu Zang from Yunnan as a member of *Heimiella* under the binomial *H. nigricans* M. Zang (Zang 1985, 1986) and more recently recombined in *Heimioporus nigricans* (M. Zang) E. Horak (Horak 2004). The re-examination of the type material preserved in the Herbarium of Cryptogams of Kunming Institute of Botany (KUN-HKAS 3224) by the Japanese authors definitely confirmed its incorrect classification and, accordingly, *H. nigricans* is here formally transferred to *Strobilomyces* with a new name (in honor of Prof. Zang), since the epithet *nigricans* was already used by Berkeley (1852) to describe a different taxon belonging to the same genus and occurring in India (type collection), Cambodia (Perreau-Bertrand 1961; Horak 1980) and possibly Australia (Watling and Gregory 1986). *S. nigricans* reported by Boedijn (1960) from Java and successfully by Corner (1972) from Malaysia seems to be another new entity.

*Strobilomyces zangii* Gelardi nom. nov.

Mycobank MB 801564

Basionym: *Heimiella nigricans* M. Zang, *Acta Botanica Yunnanica* 7 (4): 395. 1985.

non *Strobilomyces nigricans* Berkeley, *Hooker's Journal of Botany and Kew Garden Miscellany* 4: 139. 1852.

Syn.: *Heimioporus nigricans* (M. Zang) E. Horak, *Sydowia* 56: 238. 2004.

### Acknowledgements

M. Gelardi is grateful to B. Feng and Y.-J. Hao for their assistance in collecting specimens in Yunnan, and Prof. Zhu L. Yang for his hospitality and valuable suggestions. B. Feng is acknowledged for his help in the identification of the *Castanopsis* species and for providing bibliographical material concerning Chinese *Strobilomyces* species. Thanks to the State Key Laboratory of Mycology, Institute of Microbiology, and the Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences for inviting MG and for the fieldwork opportunities. A special thanks goes to E. Horak, R. Petersen, G. Simonini, and R. Watling, who also provided very useful bibliography, and whose invaluable comments and suggestions greatly helped to improve the manuscript. Caroline Hobart (Sheffield, U.K.) is thanked for improving the English text. Wu's participation was supported through a project supported by the Ministry of Science and Technology of the People's Republic of China (2008FY110300).

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